Production of Recombinant VacA of H. pylori in E. coli and Evaluation of Its Antigenicity Characteristics

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Background & Objectives: *Helicobacter pylori*, a human specific gastric pathogen and is a causative agent of chronic active gastritis, peptic ulcer and neoplasia. The vacuolating cytotoxin (vacA) is major bacterial factor involved in gastric injury. The aim of this study was to construct a recombinant protein containing antigenic region of vacA and determine its antigenicity as a vaccine candidate.

Methods: The polymerase chain reaction (PCR) was used to amplify a highly antigenic portion of vacA gene from chromosomal DNA of H. pylori. The eluted product was cloned into the prokaryotic expression vector pET32a which was digested by EcoRI and XhoI restriction endonuclease enzyme. The target protein was expressed in the E. coli BL21 (DE3) pLYsS. The genetically engineered bacteria including pET32a-vacA plasmids were induced by IPTG, the expression analyzed by SDS-PAGE; finally antigenicity was studied by western blotting using Sera of infected individual after Ni-NTA agarose resin purification.

Results: Enzyme digestion analysis, PCR and sequencing results showed that the target gene (1233 bp) was inserted correctly into the recombinant vector. The expressed protein was purified successfully via affinity chromatography using Ni-NTA resin. The data also indicated that vacA protein from Helicobacter pylori recognized by patient sera.

Conclusion: Our data showed that antigenic region of vacA protein (65kDa) can be expressed by pET32a vector in E. coli. This protein was recognized as an antigen by sera from patients suffering from H.pylori infection. Therefore, recombinant protein has similar epitopes with natural form of this antigen. Recombinant antigenic region of vacA protein also seem to be a promising antigen for protection and serologic diagnosis in human.

Keywords: Cloning, Antigenic Region; VacA Cytotoxin; *Helicobacter pylori*; Epitopes